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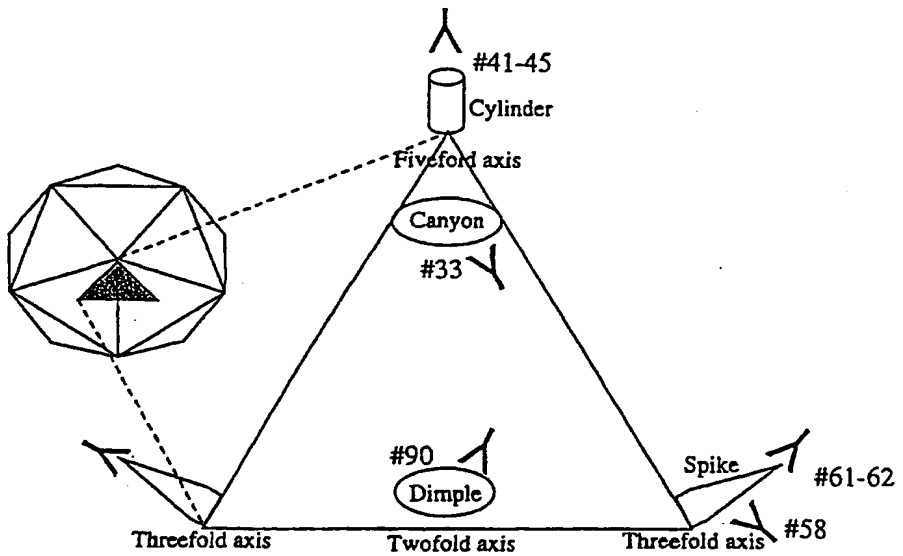

(57) Abstract: Polypeptides of virus that bind to virus antibodies or block binding of virus to mammalian cells are described. Derivatives of peptides can be less immunogenic, enhance binding to cells, render a virus tissue specific and so on. The nucleic acid sequence encoding those derivatives can be incorporated into a capsid encoding sequence to enable a virus to express such a derivative and be less immunogenic, have enhanced transduction efficiency or be tissue specific.

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VIRUS IMMUNOLOGIC DETERMINANTS

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BACKGROUND OF THE INVENTION

Modified virus, such as recombinant adeno-associated virus (AAV) vectors, are promising gene delivery vehicles. AAV, for example, is not pathogenic; the virus transduces both dividing and non-dividing cells; the virus infects a wide range of cells; and the virus integrates into the genome, which results in long term expression of the transgene. Lentivirus infects both dividing and non-dividing cells. Adenovirus is maintained as an episome and can carry large inserts.

Viral vector delivery can be obstructed by the immune response of a host to the viral component proteins. In the case of recombinant viral vectors, the primary target of the immune response is the capsid of the vector particle. For example, virus neutralizing antibodies may be generated in response to exposure to the virus or may preexist in the host because of prior exposure to wild type virus.

SUMMARY OF THE INVENTION

Virus determinants that are recognized by host antibody, such as neutralizing antibody directed to, for example, regions of the AAV capsid proteins, are mapped to

identify immunogenic sites and regions.

An object of the instant invention is to obtain and to use such immunogenic sites and regions, and functional derivatives thereof to alter the dynamics of virus binding to a cell.

The sites can be modified, for example, to render the recombinant viral vector less immunogenic or non-immunogenic to the host; to alter the tropism of the virus; to enhance binding of the virus to a cell; and to identify analogous sites in related viruses, such as, in the case of AAV, canine parvovirus.

Another object of the instant invention is to provide isolated oligopeptides that can intercede or supplant the attachment of virus and cell. Functionally equivalent derivatives thereof also are provided. The oligopeptides can be used to bind to host antibody to provide a transient tolerant or non-responsive state.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 summarizes antibody epitope mapping of AAV. Each box represents a 15 amino acid peptide sequence from AAV VP-1 starting at MAADGY... and ending with ...LTRNL. A total of 91 peptides overlapping by 5 amino acids were used. The VP-2 sequence begins with TAPGK (amino acid 149, peptide 17), and the VP-3 sequence with MATGS... (amino acid 203, peptide 25). Blackened boxes represent detection of blocking of antibody binding by that peptide in an ELISA. Blocking peptide numbers are shown for reference above and below the grid. Serum sample

designations are shown for reference to the left of the grid. Asterisks mark those sera that were positive for neutralizing antibodies.

Figure 2 summarizes the location of the immunogenic regions of AAV on the primary sequence of the capsid proteins. Shown is the amino acid sequence of the overlapping VP-1, VP-2 and VP-3 proteins that form the AAV capsid. The arrows indicate the start point of the protein sequences of VP1, 2 and 3. Identified immunogenic oligopeptides are underlined in bold and marked with the corresponding peptide designation. "Lip" denotes the insertion site of 4 amino acids that result in "low infectivity particle yield" mutants. The basic regions proposed to interact with heparin sulphate proteoglycan (HSGP) receptor are marked with a checkered line. The structural regions extrapolated from the canine parvovirus (CPV) structure are marked above the corresponding sequence. ▲: Key residues involved in determining tropism of CPV. Dashed box identifies the VFTDSE sequence recognized by CPV neutralizing dog serum.

Figure 3 is a schematic representation of the parvovirus structure, 10, adapted from Langeveld et al., infra, that shows the approximate structural locations of the immunogenic oligopeptides. The icosahedral structure (left) is composed of 60 icosahedral units (shaded triangle) formed by VP1, VP2 and VP3. The expanded triangle represents one icosahedral unit.

Figure 4 summarizes the sequences of immunogenic peptides identified by peptide blocking ELISA experiments. Overlapping sequences from two positive peptides are underlined and shown as putative epitopes, and overlapping sequences

from three juxtaposed peptides are double underlined. The shaded area corresponds to peptides that comprise a conformational epitope. Reference 23 is Hermonat et al., infra; 24 is Summerford & Samulski, infra; 17 is Tsao et al., infra; 19 is Langereld et al., infra; 18 is Wikoff et al., infra; 20 is Chang et al., infra; 21 is Parker et al., infra; and 22 is Rutledge et al., infra.

Figure 5 depicts stretches of amino acids that comprise immunologic determinants.

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of the instant invention, an immunogenic (or antigenic) oligopeptide (polypeptide or peptide) is one that is recognized and bound by an antibody or antiserum that binds to a viral vector. The immunogenic peptide also may be one that interferes with the normal functioning of the viral vector, such as binding of the virus to the cell surface. The immunogenic peptide may be an epitope, a hapten or an antigenic determinant. The oligopeptide, polypeptide or peptide of interest, those terms being considered equivalent for the purposes of the instant invention, are portions of an intact virus protein.

The phrase, amino acid, is meant to relate to the known twenty biocompatible L-amino acids that comprise proteins. The known one letter coding therefor is used herein. "Molecular Biology of the Gene", J.P. Watson et al., Benjamin Cummins, NY (1987).

Also, any one peptide described herein may be used per se as provided herein or may be modified to form an equivalent immunogenic derivative thereof. The derivative may or may not have the exact primary amino acid structure of a peptide disclosed herein so long as the derivative functionally retains the desired properties of the parent peptide disclosed herein, such as binding to an AAV antibody (or antiserum) or blocking of virus binding to a cell. The modifications can include amino acid substitution with one of the commonly known twenty amino acids or with another amino acid, with a derivatized or substituted amino acid with ancillary desirable characteristics, such as resistance to enzymatic degradation or with a D-amino acid or substitution with another molecule or compound, such as a carbohydrate, which mimics the natural confirmation and function of the amino acid, amino acids or peptide; amino acid deletion; amino acid insertion with one of the commonly known twenty amino acids or with another amino acid, with a derivatized or substituted amino acid with ancillary desirable characteristics, such as resistance to enzymatic degradation or with a D-amino acid or substitution with another molecule or compound, such as a carbohydrate, which mimics the natural confirmation and function of the amino acid, amino acids or peptide; or substitution with another molecule or compound, such as a carbohydrate or nucleic acid monomer which mimics the natural conformation, charge distribution and function of the parent peptide.

Therefore, the equivalent immunogenic derivative peptide may be comprised of amino acids, nucleotides, hydrocarbons, carbohydrates and combinations thereof. For example, a derivative may be comprised of a hydrocarbon containing substituents attached thereto.

The synthesis of a derivative can rely on known techniques of peptide biosynthesis, carbohydrate biosynthesis and so on. The various characteristics of a derivative are monitored by way of the various assays taught herein and known in the art. For example, an ELISA can be used to ensure retention of antibody binding ability.

The selection and choice of starting materials to construct the derivative is a design choice of the artisan. As a starting point, the artisan may rely on a suitable computer program to determine the conformation of a peptide of interest. Once the conformation of peptide disclosed herein is known, then the artisan can determine in a rational design fashion what sort of substitutions can be made at one or more sites to fashion a derivative that retains the basic conformation and charge distribution of the parent peptide but may possess characteristics which are not present or are enhanced over that or those found in the parent peptide.

Once candidate derivative molecules are identified, the next step is to determine which derivatives retain the requisite biologic activity of the parent peptide. That can be accomplished practicing known screening methods, some of which are taught herein. For example, an ELISA, for example, wherein virus binding antibody is immobilized on the solid phase can be used. The candidate peptides can be labeled. Alternatively, cold candidate peptides can be exposed to the solid phase antibody and then labeled virus subsequently added thereto. Alternatively, the labeled virus can be replaced with unlabeled virus and a labeled virus antibody. It should be evident that a number of permutations are possible.

As to desired characteristics of the peptide derivatives, the endpoint will

depend on the eventual use of the derivative. If the derivative is to be used as a hapten for generating virus antibody, a desirable characteristic is to have one end of the molecule carry a substituent known to be useful for conjugating molecules, for example, to a carrier molecule. Known linking molecules or substituents can be incorporated onto a peptide or peptide derivative for ready conjugation to a carrier molecule.

Another desirable feature would be resistance to peptidases. Therefore, certain amino acids of a peptide can be substituted with a replacement molecule, such as another amino acid, which would make the resulting derivative resistant to a certain peptidase.

On the other hand, in another context, serum longevity is not necessary. Instead, a transient presence in the body is desired. For example, a peptide of interest can be used to freeze the host immune system to non-reactivity to a particular virus so that a recombinant virus is able to infect and transduce target cells. Many individuals have naturally occurring antibodies directed to adenovirus and adeno-associated virus. The practice of the instant invention enables use of vectors based on those two viruses in such individuals. The subject is first exposed to a polypeptide of interest to bind to antibody specific for a particular virus and then the subject is exposed to a recombinant vector made from that virus.

The instant invention also enables repeat use of a recombinant viral vector. With viruses other than, for example, adenovirus and AAV, a host may not have naturally occurring antibodies. However, on exposure to a recombinant viral vector

carrying a therapeutic gene, a host may generate an antibody response thereto. Again, the host can be exposed to polypeptides of that virus that will bind to the specific antibody. Those polypeptides will occupy the virus binding sites of the virus antibody, thereby producing a transient tolerant state, and that host can be treated once again with a vector that is obtained from the virus from which the previously administered virus was made from.

In the case of AAV, human sera samples positive for reactivity with AAV or monoclonal antibodies directed to AAV can be used in an immunoassay, such as an ELISA, with a capsid peptide library to identify immunogenic oligopeptides that are recognized and bound by such antibodies.

Antibodies can bind to determinants composed of amino acid residues from separated portions of the secondary amino acid sequence that are spatially juxtaposed in a folded protein (conformational epitopes) or to adjacent residues on the amino acid sequence of a protein (linear epitopes). Peptides that could block antibody binding in an ELISA generally identify linear antibody epitopes. However, it is possible to distinguish lower levels of binding in a bioassay that may be suggestive of a conformational epitope. Sometimes an epitope may be sizable and contained in more that one peptide. The peptides can be configured to be non-overlapping wherein the peptides represent head-to-tail the amino acid sequence of the epitope, or the epitope can be carried by a series of two or more peptides that share a portion of the amino acids comprising same. Hence, two peptides can overlap by containing the same amino acid sequence at the tail end of the first peptide and at the head end of the second amino acid. The overlapping region represents an area of duplication between the two

peptides. The remainder of the first and second peptides is unique and together the overlapping peptides comprise a portion of the parent protein. Overlapping peptides are known in the combinatorial chemistry art.

Alternatively, a derivative may be constructed to minimize or to diminish binding thereof to the cognate antibody. Thus, the amino acid sequence thereof or the base sequence of the nucleic acid encoding the polypeptide of interest can be changed to yield a peptide derivative that does not share in the binding affinity and avidity to virus antibody as possessed by the parent peptide.

The method of identifying a peptide of interest is exemplified hereinbelow for AAV. Antibody is generated to a virus using known techniques. Viral proteins are obtained from virus stocks prepared by known methods. The viral proteins then are mapped using the antibody or serum to identify particular regions of the proteins that bind to antibody and thus represent the immunogenic sites of the virus particle. For example, the virus proteins are separated into individual species. A particular protein is fragmented, for example, by chemical or enzymatic mean as known in the art, to yield a variety of polypeptides. Then, those polypeptides are tested for binding to an antibody or serum using known methods, such as an ELISA or RIA, in a direct assay, a competitive assay and so on as known in the art.

The method can be practiced on any of a variety of viruses, particularly those that are used to develop vectors for carrying and delivering therapeutic genes. For example, murine retroviruses, human retroviruses, lentiviruses, which are considered complex retroviruses, primate retroviruses, herpesvirus, adenovirus, adeno-associated

virus and so on can be employed in the practice of the instant invention. Of those viruses, capsid proteins, envelope proteins, coat protein, essentially any protein associated with a virus that is recognized by a host immune system can be exploited in the practice of the instant invention.

Thus, the strategy will be exemplified with AAV. The AAV capsid is composed of three related proteins, VP1, VP2 and VP3 of decreasing size, present at a ratio of about 1:1:10, respectively, and derived from a single gene by alternative splicing and alternative start codon usage. Since VP-2 and VP-3 are subfragments of VP-1, a peptide library of AAV capsid protein VP-1 can be used to identify immunogenic oligopeptides of VP-2 and VP-3 as well. For example, a library composed of; for example, 15-mers overlapping by, for example, 5 amino acids, and thus containing all possible 10-mers of the 735 amino acid sequence of VP-1 can be used.

By practicing that strategy, seven regions of immunogenic sequences 15 were identified in the majority of human serum samples reactive with AAV that were tested, as depicted in Figure 1 and listed in Figures 2, 4 and 5.

Some peptides blocked antibody binding in all seven patient samples tested (e.g., peptides 4 and 5), some in the majority of patient samples (e.g., peptides 16, 17, 61 etc.) and some in only a few patient samples (e.g., peptide 33).

Several tandem peptide pairs or triplets blocked binding presumably due to a shared, overlapping epitope sequence.

The neutralizing antibody samples can be used to recognize AAV conformational epitopes.

A pool of 14 peptides (peptides 4, 5, 16, 17, 33, 61, 62, 41, 43, 44, 45, 53, 58 and 90) that blocked antibody binding in the ELISA using the human serum samples was tested to detect any relationship between and among peptides. The pool inhibited the neutralizing effect of seven different neutralizing positive sera (Ser3, Ser6, Ser7, Serl3, Ser23, Ser24 and Ser3l) to the same extent.

The peptides also reduced AAV uptake, suggesting that the series of peptides contain mimetic sequences involved in the binding of AAV to the cognate receptor thereof on the cell surface. The pool then was divided into two smaller pools of 7 peptides each. Pool I contained peptides 4, 5, 16, 17, 33, 61 and 62; and pool 2 contained peptides 41, 43, 44, 45, 53, *58,* and 90. Those combinations maintained juxtaposed peptides that likely contain a single conformation epitope or determinant within the same pool.

Pool 2 partially reversed the neutralizing effect. A control "negative pool" of 7 peptides (peptides 7, 8, 9, 10, 11, 12 and 85) showed no inhibition. Removal of peptide 90 from pool 2 had no effect on inhibition implying the core neutralizing pool of peptides to be composed of peptides 41, 43, 44, 45, 53 and 58. The same pattern was observed with five serum samples (Ser3, Ser6, Ser7, Ser23, and Ser24) and also with a neutralizing anti-AAV mouse monoclonal antibody, A20. (Wistuba et al., J. Virology 69, 5311-5319, 1995; 71, 1341-1352, 1997).

The blocking of a neutralizing monoclonal antibody suggests that the identified peptide sequences reconstitute a single conformational epitope. As shown in Figure 4, an overlap analysis and the expendability of peptide 42 point to sequences KEVT and TSTV as key residues within the conformational epitope.

The immunogenic peptides identified would be expected to be on exposed surfaces of the AAV capsid since neutralizing antibodies generally bind to the virus surface to prevent virus binding to cellular receptors and subsequent viral uptake into the cell.

There is a high structural conservation between AAV and canine parvovirus (CPV), which typifies parvovirus in general. Contact points of AAV with the receptors thereof now are identified (Summerford & Samulski, infra; Summerford et al., Nat. Med. 5, 78-82, 1999; Qing et al., Nat. Med. 5, 71-77, 1999).

The alignment of CPV VP-2 with the AAV sequence (beginning at amino acid 176) and superimposition on the CPV structure thereon (Chapman et al., Virology 144, 491-508, 1993) allow the structural location of the antigenic sites identified herein to be extrapolated between the species.

The three-dimensional structure of CPV has been determined (Tsao et al., Science 251, 1456-1464, 1991). The virus is a T=l icosahedral structure (depicted in Fig. 3) composed of 60 subunits of VP-1, VP-2 and VP-3 and is characterized by several exposed structural regions that are referred to using previously reported

nomenclature (Chapman et al., Tsao et al., supra).

Assuming AAV has a structure similar to CPV, as summarized in Figures 2 and 3, several of the B cell determinants identified correspond to exposed regions of AAV.

A "cylinder" structure protrudes from each five-fold axis and is encircled by a "canyon". Each three-fold axis also has a protruding "spike" formed by 4 loops and each two-fold axis contains a depression termed a "dimple".

Peptide 33 lies in the canyon and peptides 41-45 are located on the cylinder structure. Peptides 58, 61 and 62 are found on the spike region and peptide 90 is located at the two-fold dimple. In addition, peptide 58 binds monoclonal antibodies (Wikoff et al., Structure 2, 595-607, 1994; Langeveld et al., J. Virology 67, 765-772, 1993) and rabbit sera. Furthermore, that region contains critical residues that have been shown to determine the tropism of CPV (Chang et al., J. Virology 66, 6858-6867, 1992; Parker et al., J. Virology 71, 9214-9222, 1997) and to determine different AAV subtypes (Rutledge et al., J. Virology 72,309-319, 1998).

AAV mutants that produce 0.01 to 1% of the normal virus yield have been described (Hermonat et al., J. Virology 51, 329-339, 1984). The low infectious particle yield (lip) mutants were generated by random insertion of 8 or 9 base pair sequences which results in an in frame addition of 4 amino acids. Two of the three lip mutations map to and disrupt the peptides described herein, suggesting that those regions form surface exposed domains that are critical for virus binding and uptake.

Furthermore, one of several regions of basic amino acid motifs that have been identified and proposed to interact with the glucosaminoglycan component of HSPG of AAV (Summerford & Samulski, J. Virology 72, 1438-1445, 1998) forms part of peptides 16 and 17 (Fig. 2).

The peptides identified herein are bound by AAV neutralizing antibodies and inhibit binding of viruses to cells of a host.

As taught hereinabove, the actual amino acid sequence of any one peptide can be varied to yield an immunogenic derivative, for example, by removing one or more amino acids; adding one or more amino acids; substituting one or more amino acids; or any combination thereof. Moreover, the peptide can be mimicked by another molecule or polymer, such as a carbohydrate or a hydrocarbon. The determinative factor is whether the derivative of a specific peptide retains the distinguishing characteristics thereof; such as, binding to a virus antibody (or antiserum) or blocking binding of virus to a host cell.

A reduction in the distinguishing characteristic of up to 50% of that observed for the parent peptide when the distinguishing characteristic is desirable is tolerable in the derivative, particularly if the derivative has other desirable characteristics, such as degradation resistance. Thus, for example, if a peptide is observed to bind antibody to a certain extent, or is observed to inhibit binding of virus to a cell at a certain level at a certain concentration, a decrease of up to 50% of the observed values of the parent molecule can be found in a derivative within the scope of the instant invention

A suitable way to determine if a derivative is usable in the practice of the instant invention is to use known methods as taught herein, or equivalent methods, which demonstrate the immunogenicity and function of a peptide of the virus capsid proteins. Therefore, an immunoassay, such as an ELISA, RIA, neutralization assay and so on can be used. Also, an assay that demonstrates binding of virus to a cell can be practiced. Those such assays can afford the necessary comparison of a derivative and the parent peptide.

As taught herein, suitable derivatives are those which are found to carry desirable characteristics. For example, the oligopeptides may be manipulated to find derivatives that are less immunogenic or not immunogenic as discussed hereinabove. When such derivatives are identified, for example, the changes can be configured into the capsid coding sequence of a recombinant virus using known techniques resulting in the production of virus which will not evoke a strong or any host immune response thereto.

Also, alteration of an oligopeptide may influence the binding of a virus to a cell. A desirable characteristic would be a change that enhances binding of virus to a cell. Another desirable characteristic would be change that influences the tropism of the virus. Controlling the tropism of the virus would enable tissue-specific targeting of the viral vector. Again, once the desired change is identified, the coding sequence of the capsid proteins can be modified so that the expressed capsid proteins of the recombinant virus carry the same desirable change found in any one derivative.

Also, as noted herein, the parvoviruses share a similar structure and function.

Therefore, identification of immunogenic peptides in one species of parvovirus will enable identification of similar sites in other parvoviruses, as noted herein.

The oligopeptides of interest will find use in vitro methods, such as purification schemes. For example, oligopeptides that inhibit binding of virus to receptor can be used as competitive inhibitors to release bound virus in an adsorption-type assay. The same may apply if antibody were used as an immunoadsorbent, an oligopeptide could be used to elute bound virus from a solid support to which virus antibody is immobilized.

A polypeptide of interest can be used to block virus antibody. Therefore, a polypeptide, or derivative thereof, of interest is administered to a host by known means, such as intravenous instillation, intramuscular administration or other means, particularly by a muscular route. The polypeptide, or derivative thereof, is administered in an amount and manner that enables the preferential binding of polypeptide by antibody. The polypeptide can be configured to result in high affinity and/or high avidity binding to antibody so that the virus binding site(s) of the antibody are blocked and unable to bind virus. The polypeptide can be configured to carry substitutes that on binding to virus antibody affixes the polypeptide in the binding site, for example, by covalent reaction.

By the polypeptide binding virus antibody, the antibody is not available to bind virus. That is beneficial when recombinant virus carrying therapeutic foreign genes is administered to a host. Therefore, viruses known to stimulate a host immune response thereto can be used at lower doses and repeatedly to deliver foreign genes or defective

genes to a host by preceding administration thereof with a dosing of polypeptides to render the host non-responsive to the virus. The length of non-responsiveness need not be long-lived or permanent. The length of non-responsiveness may be transient and short-lived, of sufficient time to enable the recombinant virus to infect cells at the desired level. Therefore, in that case, there is less, if any, need for the polypeptides to have a protracted half-life in the host.

A polypeptide of interest also can be polymerized to provide a longer molecule carrying plural virus binding sites. The polypeptide monomers can be joined in tandem or regularly or irregularly interspersed with an insert linker as known in the art. The linkers can provide spatial spacing between the virus binding sites. Various linkers are known in the art and standard chemistries are used to polymerize peptides with or without intermittent spacer molecules.

A polypeptide of interest also can be attached to a carrier molecule, such as serum albumin, keyhole limpet hemocyanin, N-acetylmuramyl-alanyl-isoglutamine, monophosphoryl lipid A, BCG, Staphylococcus minnesota and so on, as known in the art using materials available in the art and commercially available, for example, from Sigma or Aldrich. The methods are not unlike conjugating a hapten on a carrier, to provide a single molecule with multiple virus antibody ligands. Known chemistries are used to attach polypeptides of interest together, to linkers or to carriers.

As taught herein, when an epitope sequence is identified, another approach would be to obtain a nucleic acid that encodes said polypeptide, modify that nucleic acid to obtain a modified epitope and that nucleic can be introduced into the virus

coding sequence, particularly substituting for the native, endogenous sequences using known methods, such as by subcloning, site directed mutagenesis, homologous recombinant and so on, so that the virus encodes, expresses and carries the modified epitope. If the polypeptide is configured so that the polypeptide no longer binds to a particular antibody, then the virus now no longer expresses a site that is recognized by the antibody that bound to the native, endogenous site.

The peptides, and particularly certain immunogenic derivatives thereof; may find use in vivo. Also, the sequence of modified peptides can be incorporated into the capsid sequence of a recombinant virus by subcloning a polynucleotide encoding such a modified peptide into the nucleic acid encoding a capsid protein. The polynucleotide can replace the sequence found in the wild-type capsid nucleic acid. Methods for manipulating pieces of nucleic acids are known. Methods for making recombinant virus are known in the art. Moreover, methods for administering peptides or virus are known in the art. The amounts of peptides or viral vectors to be administered to a host in need of treatment will have been determined for the unmodified virus. Because the peptides of the instant invention, if the sequences therefor are incorporated into a virus, would be, for example, less immunogenic, a lower dosage can be used. An artisan would determine the appropriate new dosage by extrapolating from pre-clinical data or clinical data. Regarding the dosing of peptides, again the artisan would follow accepted methods of extrapolating from pre-clinical and clinical studies. As some derivatives may be stable, that is, resistant to degradation in the host, the long term dosing would have to be adjusted to take those characteristics into account. The amount of peptide or virus in the host can be determined by sampling, for example, a blood specimen or a tissue biopsy, and determining the levels thereof therein using known techniques, such

as those taught therein.

Preclinical and clinical data are used in formulating a range of dosing for human use. The dose may vary depending on the form used and the route of administration. The artisan will know how to make necessary adjustments.

Pharmaceutical compositions comprising virus and polypeptides may be formulated as known using physiologically acceptable carriers, diluents or exicipients. For example, in solution, the diluent can be a pharmaceutically acceptable saline solution with preservatives as needed.

The virus and polypeptide preparations are formulated for administration by any of a variety of routes, such as, inhalation, oral, buccal, parenteral or rectal administration.

For administration by inhalation, the virus and polypeptide can be delivered as an aerosol spray from pressurized packs or a nebulizer, with the use of a suitable propellant.

For oral administration, the pharmaceutical compositions may take the form of for example, tablets, lozenges or capsules prepared by conventional means with pharmaceutically acceptable excipients, such as binding agents; fillers; lubricants; glidants; disintegrants; or detergents. The tablets may be coated.

Liquid preparations may take the form of; for example, solutions, syrups or

suspensions, or a dry product for constitution with water or other suitable vehicle before use. The liquid preparations can contain pharmaceutically acceptable additives such as suspending agents; emulsifying agents; non-aqueous vehicles; and preservatives. The preparations may also contain buffer salts, flavoring, coloring and sweetening agents.

Preparations for oral administration may be suitably formulated to provide controlled release of the active compound.

The virus and polypeptide may be formulated for parenteral administration by injection, for example, by bolus injection or infusion. Formulations for injection may be presented in unit dose, for example, in ampoules or in multidose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles as needed, and may contain additives such as suspending, stabilizing and dispersing agents. Alternatively, the active ingredient may be in a powder or a lyophilized form for constitution with a suitable vehicle, for example, sterile pyrogen-free water, before use.

The virus and polypeptide also may be formulated for long term release. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the therapeutic compounds may be formulated with suitable deposition material, for example, an emulsion.

The compositions may, if desired, be presented in a pack or dispenser device

which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

The invention now will be exemplified in the following non-limiting examples.

EXAMPLE 1

Construction and production of AAV vectors

AAV vectors expressing green fluorescent protein (GFP) (Klein et al., Exp. Neurol. 150, 183-194, 1998), β-galactosidase (McCown et al., Brain Res. 713, 99-107, 1996) and hFIX were constructed and generated using known techniques, such as taught in Snyder et al., (Nat. Genet. 16, 270-272, 1997). Titers were determined by dot blot analysis.

EXAMPLE 2

Detection of anti-AAV antibodies using ELISA

Ninety-six well MaxiSorp flat surface Nunc-Immuno plates were coated with 5×10^7 particles of AAV in 1000 µl/well of 0.1 M carbonate buffer pH 9.6, incubated overnight at 4°C and washed twice with washing buffer from an AMPAK amplification kit (DAKO, Carpenteria, CA). After blocking with 3% BSA in washing buffer for

2 hours at room temperature, the plates were washed once and incubated for 1 hour at room temperature with donor serum at 1:100 dilution in washing buffer, 1% BSA in a total volume 100 µl/well. Next, the plates were washed 5 times and AP conjugated mouse anti-human antibodies (Zymed, San Francisco, CA) were added at 1:800 dilution in washing buffer, 1% BSA, 100 µl/well. The plates were incubated for 1 hour at room temperature and washed with washing buffer 4 times. For color development and further amplification of the signal, the AMPAK amplification kit was used. Absorbance was measured at 490 nm.

EXAMPLE 3

Detection of neutralizing anti-AAV antibodies

293 cells were seeded in a 24 well plate at a density of 1 x 10^5 cells per well, in 1 ml of IMDM media (JRH). The cells were allowed to adhere for 2 hours at 37°C. The media then was removed by aspiration before 6 x 10^6 particles of adenovirus d1309 (Ferrari et al., J. Virology 70, 3226-3234, 1996), were added in a final volume of 200 µl per well. The cells were incubated further at 37°C for 1 hour and then washed twice in the same media before the following mix was added. AAV-GFP (1 µl = 5 x 10^8 total particles or 9 x 10^6 transducing units) virus was incubated with serum sample diluted in PBS for 2 hours at 4°C in a total volume of 25 µl. The final dilution of the test serum was 1:100 or 1:1000. The mix was added to the washed cells in a final volume of 200 µl, and incubated for 1 hour at 37°C. About 400 µl of media then were added to each well and cells were incubated overnight. Cells were collected, washed in PBS/BSA (1%), and analyzed by FACS. The % inhibition was calculated using a "no

antibody" control sample as a reference. Another control was anti-AAV guinea pig sera that showed maximal inhibition.

EXAMPLE 4

Epitope mapping of anti-AAV antibodies

A set of 91 overlapping peptides (15mers) spanning the entire 735 amino acid AAV-VP1 capsid protein sequence (Genbank # AF043303) were synthesized using the PIN synthesis strategy (Chiron Mimotopes, Clayton, Australia). The peptide sequences overlap by 5 amino acids thus generating all possible 10mers of VP-1. Two control peptides also were synthesized to verify purity and assess yield. Peptides were resuspended in PBS at a concentration of 5 mg/ml and stored at -20°C.

ELISA analysis was performed in the presence of 1 µl (corresponding to a final concentration of approximately 20 µM) of individual peptides or 10 µl peptide pools which were present at the antibody incubation stage. Similarly, 1 µl of each peptide was added to the *25* µl antibody-AAV-GFP mix in the neutralizing assay to assess the ability to block the binding of neutralizing antibodies to AAV-GFP.

All references cited herein are incorporated by reference in entirety.

It will be readily evident to the artisan that various changes and modifications can be made to the teachings herein without departing from the spirit and scope of the instant invention.

We claim:

1. An isolated polypeptide portion of a virus protein, or a derivative thereof, wherein said polypeptide and said derivative bind to antibody specific for said virus or inhibit binding of said virus to a cell.

2. The polypeptide or derivative of claim 1, wherein said virus is adenovirus.

3. The polypeptide or derivative of claim 1, wherein said virus is adeno-associated virus.

4. The polypeptide or derivative of claim 1, wherein said virus is a retrovirus.

5. The polypeptide or derivative of claim 1, wherein said derivative is a polypeptide.

6. The polypeptide or derivative of claim 1, wherein said derivative is synthetic.

7. A nucleic acid encoding said polypeptide or derivative of claim 5.

8. A composition comprising the polypeptide or derivative of any one of

claims 1-6 and a carrier.

9. The composition of claim 8 comprising two or more polypeptides.

10. The composition of claim 8, wherein said two or more polypeptides have the same sequence.

11. The composition of claim 8, wherein said two or more polypeptides have overlapping sequences.

12. The composition of claim 8, wherein said two or more polypeptides have distinct sequences.

13. A virus particle comprising the polypeptide or derivative or any one of claims 1-6.

14. A nucleic acid that encodes a derivative of a polypeptide of a virus, wherein said polypeptide binds to antibody specific for said virus, and wherein said derivative does not bind to said antibody.

15. The nucleic acid encoding said derivative of claim 14, wherein said virus is adenovirus.

16. The nucleic acid encoding said derivative of claim 14, wherein said virus is adeno-associated virus.

17. The nucleic acid encoding said derivative of claim 14, wherein said virus is a retrovirus.

18. A polypeptide encoded by the nucleic acid of any one of claims 14-17.

19. A virus particle comprising the polypeptide of claim 18.

20. A pharmaceutical composition comprising a polypeptide or derivative of any one of claims 1-6 and a pharmaceutically acceptable carrier, excipient or diluent.

21. A pharmaceutical composition comprising a polypeptide of claim 18 and a pharmaceutically acceptable carrier, excipient or diluent.

22. A method of inhibiting binding of a virus antibody to said virus comprising exposing said virus antibody to the polypeptide or derivative of any one of claims 1-6.

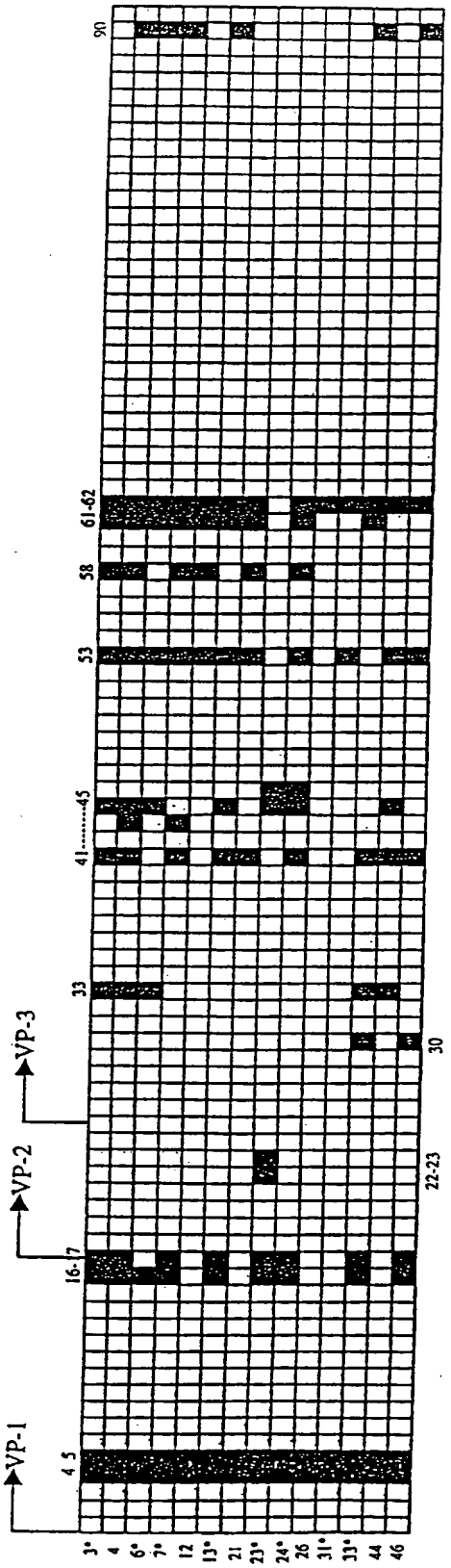
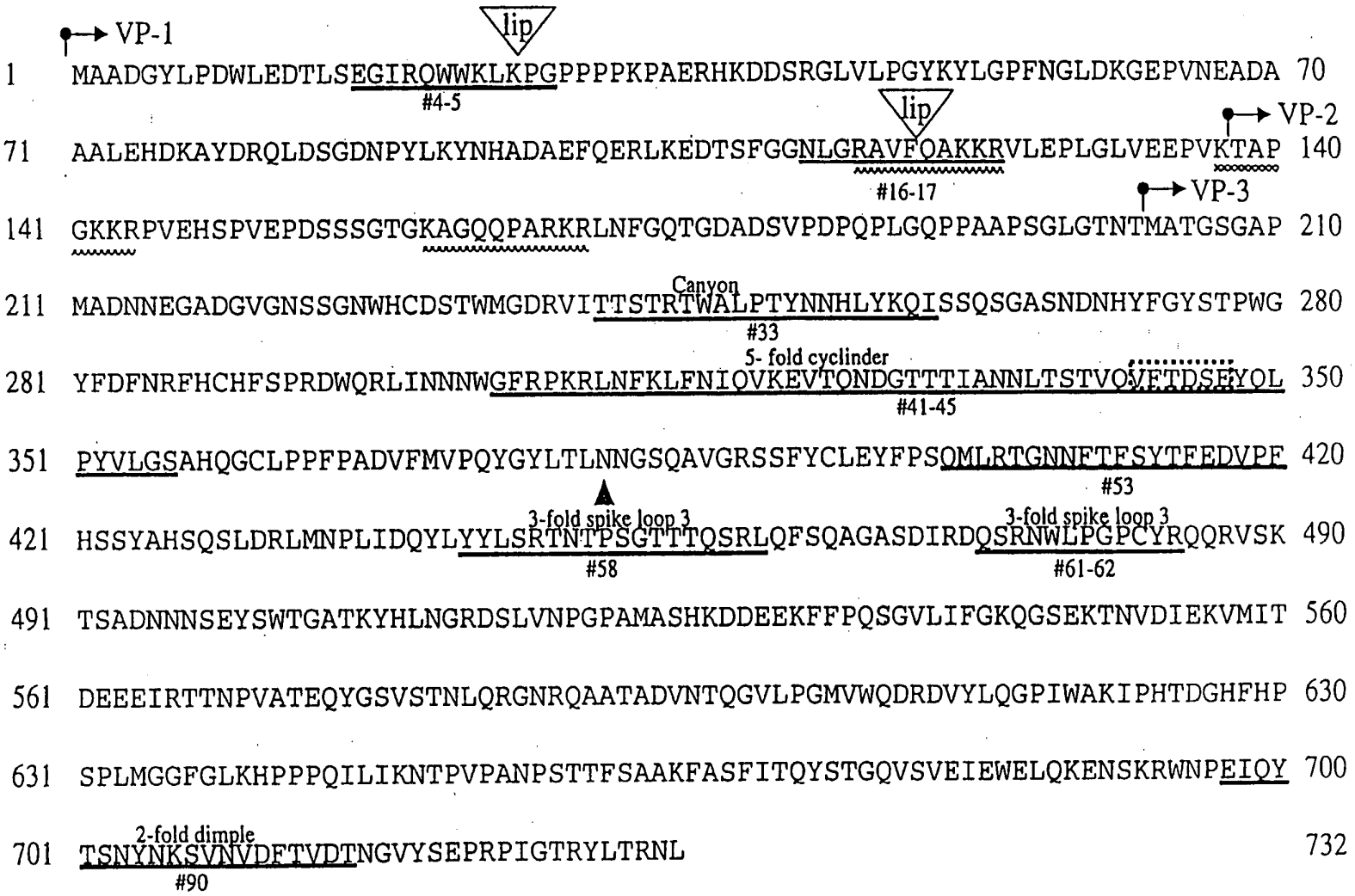

90
61-62
58
53
41.....45
33
30
22-23
16-17
4 5
VP-1
VP-2
VP-3
3*
4
6*
7*
12
13*
21
23*
24*
26
31*
33*
44
46

Figure 1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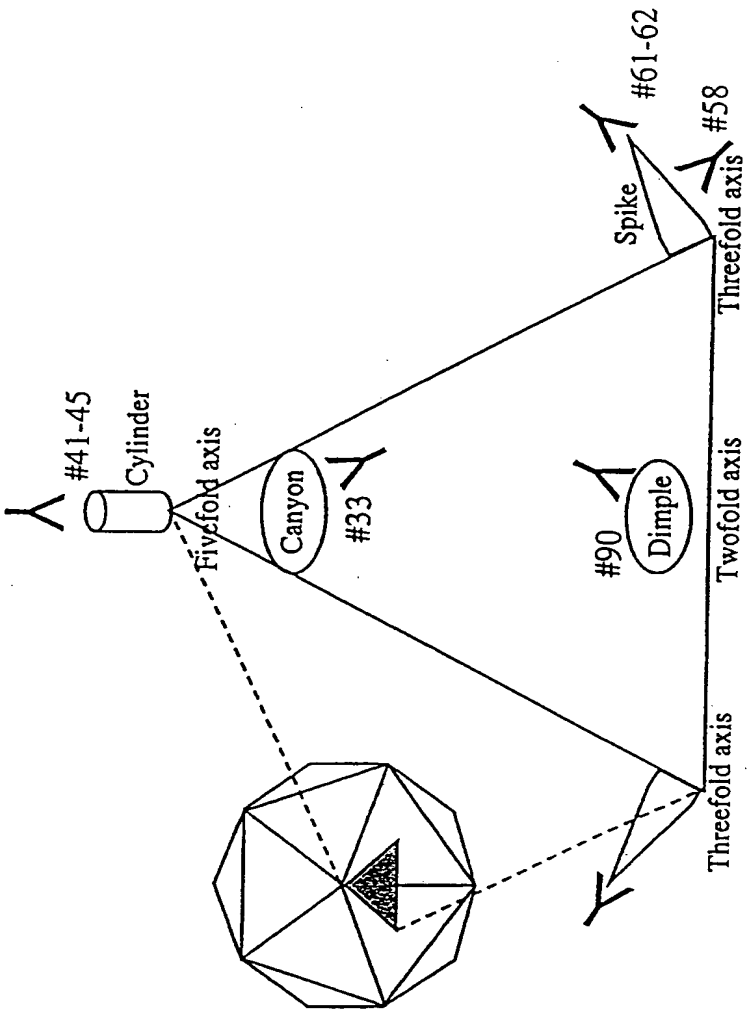
lip
1 MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFNGLDKGEPVNEADA 70
#4-5
lip
VP-2
71 AALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAP 140
#16-17
VP-3
141 GKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGTNTMATGSGAP 210
Canyon
211 MADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWG 280
#33
5- fold cyclinder
281 YFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQL 350
#41-45
351 PYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPF 420
#53
3-fold spike loop 3
3-fold spike loop 3
421 HSSYAHSQSLDRLMNPLIDQYLYYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSK 490
#58
#61-62
491 TSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMIT 560
561 DEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHP 630
631 SPLMGGFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQY 700
2-fold dimple
701 TSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL 732
#90

Figure 2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Cylinder
Fivefold axis
Canyon
#33
#90
Dimple
Twofold axis
Threefold axis
Spike
#61-62
#58
Threefold axis

Figure 3

Mapped peptide	Putative epitope	Supporting evidence
DWLEDTLSEGIRQWWKLKPG EGIRQWWKLKPGPPPPKPAE	EGIRQWWKLKPG	• Site of "lip" insertion[23].
KEDTSFGGNLGRAVFQAKKR NLGRAVFQAKKRVLEPLGLV	NLGRAVFQAKKR	• Site of "lip" insertion[23]. • RAVFQAKKR proposed to bind HSPG[24].
TTSTRTWALPTYNNHLYKQI		• Corresponds to "canyon" floor[17].
GFRPKRLNFKLFNIQVKEVT KEVTQNDGTTTIANNLTSTV	KEVT	• Corresponds to "cylinders" on capsid surface[17].
TTTIANNLTSTVQVFTDSEY TSTVQVFTDSEYQLPYVLGS	TSTV	• cPV neutralizing dog sera binds to VP1DSB[19]. • Corresponds to "spike" region[17]. • Antigenic region bound by mAbs A3B10B8, 4AG6, 3C10 and rabbit sera[19]. • Tropism determining region in cPV[20,21] and AAV[22].
QMLRTGNNFTFSYTFEDVPF		
YLYYLSRTNTPSGTTTQSRL		
AGASDIRDQSRNWLPGPCYR QSRNWLPGPCYRQQRVSKTS	QSRNWLPGPCYR	• Corresponds to "spike" region[17].
EIQYTSNYNKSVNVDFTVDT		• Corresponds to "dimple" region[17].

Figure 4

EGIRQWWKLKPG

NLGRAVFQAKKR

TTSTRTWALPTYNNHLYKQI

GFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGS

QMLRTGNNFTFSYTFEDVPF

YLYYLSRTNTPSGTTTQSRL

QSRNWLPGPCYR

EIQYTSNYNKSVNVDFTVDT

Figure 5